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PRINCIPAL INVESTIGATOR: Michael A. White, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas
Southwestern Medical Center
Dallas, Texas 75390-9105

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6. AUTHOR(S)
Michael A. White, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Texas Southwestern Medical Center
Dallas, Texas 75390-9105

E-Mail: Michael.white@utsouthwestern.edu

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13. ABSTRACT ***(Maximum 200 Words)***

While not expressed in most adult human tissues, high levels of telomerase activity are present in most cancers. Expression of telomerase in many primary cell cultures is sufficient to bypass normal senescence. Cellular senescence can act as a terminal growth-control checkpoint, preventing the progression of pre-cancerous cells to malignancy. Blockade of telomerase activity in breast neoplasias should reintroduce this checkpoint, resulting in replication-dependent senescence of proliferating cells. Therefore, telomerase antagonists could potentially control growth and metastasis of residual cancer cells after surgery and chemotherapy. We have identified the molecular chaperones p23, and hsp90 as proteins that bind to the catalytic subunit of telomerase. Blockade of this interaction inhibits assembly of active telomerase in vitro. Therefore, we anticipate that small molecules that bind hTERT and prevent association with p23 will act as telomerase antagonists in cells. We have used selective screens of a library of structurally constrained poly-peptides for those that disrupt hTERT/p23 complexes by binding directly to hTERT. Peptides isolated from this screen will be tested for inhibitory effects on telomerase activity, telomere maintenance, and proliferative capacity. We predict that introduction of telomerase antagonists in breast cancer cells will result in the replication-dependent senescence of these cells.

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Introduction: One of the earliest events associated with the multistage pathogenesis of breast cancer is the activation of telomerase. Telomerase is a reverse transcriptase that elongates the ends of chromosomes and is required to prevent replicative senescence in proliferative cells. While not expressed in most adult human tissues, high levels of telomerase activity are present in most cancers. Ectopic expression of telomerase in many primary cell cultures is sufficient to bypass normal senescence. Cellular senescence can act as a terminal growth-control checkpoint, preventing the progression of pre-cancerous cells to malignancy. Blockade of telomerase activity in breast neoplasias should reintroduce this checkpoint, resulting in replication-dependent senescence of proliferating cells. Therefore, telomerase antagonists could potentially control growth and metastasis of residual cancer cells after surgery and chemotherapy. The telomerase holoenzyme is a multi-subunit complex consisting of a reverse transcriptase catalytic core (hTERT) and an RNA template component (hTR) together with other unknown accessory factors. We have recently identified critical protein/protein interactions in the telomerase holoenzyme required for catalytic activity. Here we are developing telomerase antagonists that target these interactions to functionally disrupt the holoenzyme. We have identified the molecular chaperones p23, and hsp90 as proteins that bind to the catalytic subunit of telomerase. Blockade of this interaction inhibits assembly of active telomerase in vitro. Also, a significant fraction of active telomerase from cell extracts is associated with p23 and hsp90. Consistent with in vitro results, inhibition of hsp90 function in cells blocks assembly of active telomerase. The association of human p23 with hTERT can be monitored in the yeast two hybrid system. This allows selective genetic screens to be developed for isolation of factors that specifically disrupt p23/hTERT complexes. We have shown that inhibition of hTERT/p23 association inhibits production of active telomerase. Therefore, we anticipate that small molecules that bind hTERT and prevent association with p23 will act as telomerase antagonists in cells. Our objective is to use selective screens in yeast to isolate such molecules. We are screening a library of structurally constrained poly-peptides for those that disrupt hTERT/p23 complexes by binding directly to hTERT. Peptides isolated from this screen will be tested for inhibitory effects on telomerase activity, telomere maintenance, and proliferative capacity. We predict that introduction of telomerase antagonists in breast cancer cells will result in the replication-dependent senescence of these cells.

Body:

As outlined in our original "Statement of Work", we have focused research in the past year on Task1.

Task 1 (months 1-15). Isolate a pool of peptide aptamers that bind hTERT such that they block association with p23.

Progress: We have screened 6 million transformants from a library with a complexity of $1X10^8$. Several hundred positives were isolated and shuttled into the secondary screen. Out of this, 3 clones were isolated encoding aptamers that specifically interact with hTERT and inhibit the hTERT/p23 interaction (as assessed by two-hybrid). These 3 clones are currently being shuttled into E. coli expression vectors for production of recombinant protein. This protein will be used to begin Task 2.

Future work: We have isolated 3 aptamers from a sampling of 6% of the total complexity of our library. We are continuing these screens to isolate additional aptamers (we would ideally like to isolate at least 30 to aid identification of conserved motifs). We have scaled up the primary screen to derive 10 million transformants per yeast transformation per week. This should produce an additional 30 aptamers in six weeks.

Task 2 (months 15-24). Identify those peptide aptamers isolated in task1 that will inhibit production of active telomerase in vitro.

Progress: As anticipated in our original "Statement of Work", we are now in position to begin this task using the aptamers we have isolated from Task1.

Key Research Accomplishments:

In year 1, we have isolated 3 peptide aptamers that specifically associate with hTERT to block interaction with p23.

Reportable Outcomes:

None.

Conclusions:

The reagents that are being developed will be useful in examining the biological consequences of inhibiting telomerase activity in human breast cancer cells. These reagents will have potential clinical applications to breast cancer therapy and prevention.